UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

008581

SEP 12 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

**MEMORANDUM**

**SUBJECT** **Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid:** Industry Task Force II on 2,4-D Research Data Flagged in Registration Standard for Priority Review.

**FROM:** Jess Rowland, M.S, Toxicologist Jess Rowland 9/9/91
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Toxicology Branch II (HFAS)
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**STUDY IDENTIFICATIONS:** Case No: 818715 Submission No: 8397263

HED Project No.1-1443 Caswell No. 315-O

**Registrant:** Industry Task Force II on on 2,4-D Research Data

**ACTION REQUESTED:** Review of a subchronic toxicity study for 2,4-D DMA salt, identified for priority review in the registration standard for 2,4-D.

1718

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**SUMMARY:** Male and female Fischer-344 rats were fed diets containing the Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid at 0, 1.2, 18.1, 120.0, and 361.0 mg/kg/day for 13 weeks. Treatment did not cause any adverse effects at 1.2 or 18.1 mg/kg/day, but did induce decreases in mean body weight, body weight gain, and food consumption, and alterations in some of the hematology and clinical chemistry parameters at 120.0 mg/kg/day.

The highest dietary dose level of 361.0 mg/kg/day was associated with weight loss, reduced food consumption, alterations in hematology and clinical chemistry parameters, changes in various organ weights, and histopathological changes that included bilateral retinal degeneration and cataract formation (females); centrilobular hepatocellular hypertrophy (females); atrophy of the testes (males); hypertrophy of thyroid follicular cells, and brush order loss in proximal tubular cells in the kidney (males and females); and hypoplasia of the spleen (females) and bone marrow (males and females).

Under the conditions of this study, a No-Observable-Effect Level (NOEL) of 18.1 mg/kg/day and a Lowest-Observable-Effect Level (LOEL) of 120.0 mg/kg/day are established. The LOEL is based on decreases in mean body weight, body weight gain and food consumption, and alterations in hematology and clinical chemistry paramters observed at 120 mg/kg/day.

**CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

**PRIMARY REVIEWER:** Jess Rowland, M.S, Toxicologist 7/9/91
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**SECONDARY REVIEWER:** K. Clark Swentzel, Section Head
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**DATA EVALUATION REPORT**

008581

**STUDY TYPE:** 90-day Feeding-Rodent **GUIDELINE:** 82-1(a)

**TOX.CHEM. No.:** 315-O **MRID No.:** 418967-02

**HED Project No.** 1-1443 **Registrant:** Industry Task Force II

**TEST MATERIAL:** Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid (2,4-D DMA)

**STUDY IDENTIFICATION:** HLA Study No. 2184-113

**TESTING LABORATORY:** Hazleton Laboratories America, Inc.

**TITLE OF REPORT:** Subchronic Toxicity Study in Rats with the Dimethylamine Salt of 2,4-Dichlorophenoxyacetic Acid.

**REPORT AUTHOR:** Gene E. Schulze, Ph.D

**REPORT DATE:** April 20, 1991

**SUMMARY:** Male and female Fischer-344 rats were fed diets containing the Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid at 0, 1.2, 18.1, 120.0, and 361.0 mg/kg/day for 13 weeks. Treatment did not cause any adverse effects at 1.2 or 18.1 mg/kg/day, but did induce decreases in mean body weight, body weight gain, and food consumption, and alterations in some of the hematology and clinical chemistry parameters at 120.0 mg/kg/day. The highest dietary dose level of 361.0 mg/kg/day was associated with weight loss, reduced food consumption, alterations in hematology and clinical chemistry parameters, changes in various organ weights, and histopathological changes that included bilateral retinal degeneration and cataract formation (females); centrilobular hepatocellular hypertrophy (females); atrophy of the testes (males); hypertrophy of thyroid follicular cells, and brush order loss in proximal tubular cells in the kidney (males and females); and hypoplasia of the spleen (females) and bone marrow (males and females).

Under the conditions of this study, a NOEL of 18.1 mg/kg/day and a LOEL of 120.0 mg/kg/day are established. The LOEL is based on decreases in mean body weight, body weight gain and food consumption and alterations in hematology and clinical chemistry parameters observed at 120 mg/kg/day.

**CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

## I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the subchronic toxicity of **Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid** following dietary administration to rats.

## II. MATERIALS AND METHODS

### 1. Test and Control Articles

Test Chemical Name: Dimethylamine Salt of 2,4-Dichlorophenoxyacetic acid.
Purity: 66.18%
Lot No.: 04FD31349
Description: Brown liquid.
Acid equivalent: 55.5%
Vehicle: Acetone, reaction grade, Mallenckrodt
Lot. No: 2440 KDMS

### 2. Test Animals

Species: Rats
Strain: CDF (F-344)/CrljBR Fischer-344 VAF/Plus
Sex: Males and females
Age: Approximately 6 weeks at initiation
Weight at initiation: 123.5-142.4 g (M); 92.0-104.2 g (F)
Identification: Tail tattoo.
Acclimation: Approximately 2 weeks
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Chow #5002.
Water: Tap water ad libitum
Environment: Temperature- 70 - 78° F; Humidity- 45 - 70 %;
Light/dark cycles: 12 hr light/12 dark cycle

### 3. Study Design

| Group No. | Treatment | No. of Animals | | Dose Level mg/kg/day |
|---|---|---|---|---|
| | | Males | Females | |
| 1 | Control | 10 | 10 | 0 |
| 2 | Low | 10 | 10 | 1.2 |
| 3 | Mid-1 | 10 | 10 | 18.1 |
| 4 | Mid-2 | 10 | 10 | 120.0 |
| 5 | High | 10 | 10 | 361.0 |

4. Test Article Formulation and Analyses

The purity (66.18%) of 2,4 D DMA was adjusted to 100% active compound. The test article was stirred with a spatula and put on a shaker for approximately 1 hour to ensure an apparently homogeneous liquid state.

For each dose level, an appropriate amount of the test article was weighed to which an appropriate volume of acetone was added, and the test article/acetone mixture was then stirred on a magnetic stirrer for approximately 2 minutes or until an apparently homogeneous mixture was achieved. Final diets were prepared by mixing the test article/acetone mixture with additional powdered feed in a Hobart mixer for 20 minutes. The control diet was prepared in the same manner as the test diets using acetone only at the same level utilized in the test diets. Fresh diets were prepared weekly and reserve samples of each mixed batch were taken on the day of preparation and stored in a freezer.

Concentration analyses of the low and high dose levels were performed prior to initiation of treatment based on historic body weight and food consumption data. Homogeneity was determined of samples from the top, middle, and bottom of each diet mix. Stability analyses were performed on days 0, 7, and 14. Freshly prepared samples were collected from each dose level of the mixed for Weeks 1-4, 8, and 12 and were analyzed for verification of concentration.

5. Treatment

Rats were fed the control and test diets 7 days per week for at least 13 weeks. The oral route of administration was chosen because it is the route of potential human exposure.

6. Experimental Procedures

Mortality and moribundity checks were performed twice daily. Cageside observations for clinical signs of toxicity were performed once daily. Body weights were measured prior to initiation and once weekly. Food consumption was recorded weekly and physical examinations were performed weekly at the body weight intervals. Ophthalmologic examinations were conducted prior to initiation and during Weeks 11 and 13.

Blood and urine were collected from all animals during Weeks 6 and 14 for hematology, clinical chemistry and urinalysis. The checked (x) parameters were determined.

Hematology

| | |
|---|---|
| x Hematocrit (HCT)[a] | x Leukocyte count (WBC)[a] |
| x Hemoglobin (HGB)[a] | x Platelet count[a] |
| x Erythrocyte count (RBC)[a] | x Leukocyte differential[a] |
| Mean corpuscular HGB (MCH) | Mean corpuscular HGB Concentration (MCHC) |
| Mean corpuscular volume (MCV) | Blood clotting measurements |
| x Corrected leukocyte count (COR WBC) | x Cell morphology |

Clinical Chemistry

| Electrolytes: | Other |
|---|---|
| x Calcium[a] | x Albumin[a] |
| x Chloride[a] | x Blood creatinine[a] |
| Magnesium[a] | x Blood urea nitrogen[a] |
| x Phosphorus[a] | Cholesterol[a] |
| x Potassium[a] | x Globulins |
| x Sodium | x Glucose[a] |
| | x Total bilirubin[a] |
| Enzymes: | x Total serum protein[a] |
| | Triglycerides |
| x Alkaline phosphatase | Serum protein electrophoresis |
| x Alanine aminotransferase (SGPT)[a] | x Triiodothyronine ($T_3$ |
| x Aspartate aminotransferase (SGOT)[a] | x Thyroxine ($T_4$) |
| Cholinesterase[b] | |
| x Creatinine phosphatase[a] | |
| Lactic acid dehydrogenase | |
| Gama glutamyl transferase | |

Urinalysis

| | |
|---|---|
| x Appearance[c] | x Bilirubin[c] |
| x Specific gravity[c] | x Occult blood[c] |
| x pH | x Urobilinogen |
| x Protein | x Glucose[c] |
| x Ketones[c] | Microscopic examination of sediment[c] |

[a] Required for subchronic and chronic studies.
[b] Required only for organophosphates and carbamates.
[c] Required for chronic studies.

7. Termination

After 13 weeks of treatment, all surviving animals were weighed, anesthetized with sodium pentobarbital and exsanguinated. Necropsies were performed on each animal, all gross pathological changes were recorded, and the following organs were weighed.

| | | | | |
|---|---|---|---|---|
| Adrenals | Brain | Heart | Kidneys | Liver |
| Ovaries | Thymus | Thyroid | Pituitary | Testes |

8. Histopathology

The checked (X) tissues from all animals were trimmed and processed for histopathological evaluation.

| Digestive System | Respiratory System |
|---|---|
| x Salivary glands[a] | Trachea[a] |
| x Esophagus[a] | x Lung[a] |
| x Duodenum[a] | Pharynx[e] |
| x Jejunum[a] | Larynx[e] |
| x Cecum[a] | Nose[e] |
| x Colon[a] | |
| x Rectum[a] | Cardiovascular/Hemo.System |
| x Liver[ac] | |
| x Pancreas[a] | x Aorta (thoracic)[a] |
| Gall bladder[ab] | x Heart[a] |
| | x Bone marrow[a] |
| Neurological System | x Lymph nodes[a] |
| | x Spleen[a] |
| x Brain[ac] | x Thymus[a] |
| x Pituitary[a] | |
| Peripheral nerve[ab] | Urinogenital System |
| Spinal cord (3 levels)[ab] | |
| x Eyes (optical nerve)[ab] | x Kidneys[ac] |
| | x Urinary bladder[a] |
| Glandular System | x Testes[ac] |
| | x Epididymides |
| x Adrenals[a] | x Uterus[a] |
| Lacrimal glands[c] | x Ovaries[ac] |
| x Parathyroids[ad] | Others |
| x Thyroids[ac] | |
| | x All gross lesions and masses |
| | x Skeletal muscle[a] |

a. Required for subchronic and chronic studies.
b. In subchronic studies examined only if indicated by toxicity or target organ involvement.
c. Organ weights required in subchronic and chronic studies.
d. Organ weights required for nonrodent studies.
e. Required for chronic inhalation study.

9. Statistical Analyses

Mean absolute body weight, body weight change, mean weekly food consumption, total food consumption, clinical pathology data (with the exception of urinalyses and cell morphology), and organ weight data of the control group were compared statistically to the data from the compound-treated groups.

10. Quality Assurance

The study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of Hazleton Labs, and the Study Protocol. A quality assurance statement was signed and dated April 9, 1991.

## III. RESULTS

### 1. Analysis of Diet Mix

Concentration analyses summarized below showed that in general, the mean concentration was within 10% of target level except for the high dose mix at Week 12 (74%).

| Group / Sex | Target Level (ppm) | Target Level (Mean%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Week 1 | Week 2 | Week 3 | Week 4 | Week 8 | Week 12 |
| 2 / M | 16 | 103 | 105 | 92.1 | 91.5 | 100 | 107 |
| 2 / F | 14 | 110 | 99.9 | 102 | 108 | 101 | 94.7 |
| 3 / M | 241 | 97.5 | 100 | 97.4 | 104 | 102 | 102 |
| 3 / F | 211 | 100 | 101 | 98.9 | 99.7 | 105 | 101 |
| 4 / M | 1621 | 106 | 86.4 | 101 | 102 | 105 | 100 |
| 4 / F | 1463 | 106 | 101 | 96.7 | 99.9 | 97.9 | 94.4 |
| 5 / M | 5041 | 106 | 101 | 96.9 | 101 | 101 | 74.0 |
| 5 / F | 4731 | 108 | 107 | 98.1 | 103 | 104 | 97.9 |

Homogeneity analysis indicated the diet mix to be homogeneous with target levels ranging from 77 to 124% (mean, 95.5%) in the top, middle, and bottom samples at the low dose and ranging from 88 to 94% (mean, 92%) in the top, middle, and bottom samples at the high dose.

Stability analyses of samples stored at room temperature demonstrated the test material to be stable with recovery of 98% and 99% at the low and high dose, respectively after 7 days, and 95% and 100%, respectively after 14 days.

### 2. Survival

Except for one female that died at the high dose (361 mg/kg/day) that died following the Week 6 blood sample collection, no mortality occurred during the study.

2. Clinical Observations

Treatment-related clinical signs of toxicity were limited to opaque eyes observed in 6/10 females at 361.0 mg/kg/day; other frequently seen signs (urine stains, alopecia, and lacrimation) did not appear to be dose- or compound-related.

3. Body Weights and Body Weight Changes

Mean body weight data are summarized in Tables 1 and 2 for males and females, respectively. Statistically significant ($p < 0.05$) reduction in mean body weight was observed for males and females at 361 mg/kg/day at Weeks 6 and 13, and for females at 120 mg/kg/day at Week 6. A significant monotonic negative trend was seen for the males at Week 13.

**Table 1. Mean Body Weights and S.D (G) for Male Rats.**

| Dose Level (mg/kg/day) | Week: 0 | Week: 6 | Week: 10 | Week: 13[a] |
|---|---|---|---|---|
| 0 | 133.3 ± 4.1 | 250.2 ± 5.8 | 293.0 ± 11.2 | 305.9 ± 12.2 |
| 1.2 | 132.5 ± 5.0 | 242.3 ± 16.3 | 284 ± 21.5 | 301.0 ±21.0 |
| 18.1 | 128.9 ± 3.0 | 243.3 ± 10.4 | 285.0 ± 12.3 | 301.0 ±12.1 |
| 120.0 | 133.9 ± 3.8 | 248.3 ± 11.5 | 290.1 ± 13.0 | 307 ± 14.5 |
| 361.0 | 133.5 ± 5.1 | **196.6* ± 12.8** | 219.6 ±17.5 | **234.0 ± 18.0*** |

* Significantly different from control value at $p \leq 0.05$.
a Significant negative monotonic trend, $p \leq 0.05$

**Table 2. Mean Body Weights and S.D (G) for Female Rats.**

| Dose Level (mg/kg/day) | Week: 0 | Week: 6 | Week: 10 | Week: 13 |
|---|---|---|---|---|
| 0 | 99.2 ± 2.5 | 140.0 ± 5.5 | 162.1 ± 8.1 | 169.4 ± 9.1 |
| 1.2 | 99.1 ± 3.9 | 143.0 ± 9.8 | 167.0 ± 11.7 | 174.0 ± 12.5 |
| 18.1 | 99.5 ± 2.0 | 142.6 ± 1.9 | 165.1 ± 3.3 | 169.5 ± 6.9 |
| 120.0 | 101.1± 2.7 | 132.6 ± 5.6 | 154.4 ± 7.0 | 162.5 ± 6.6 |
| 361.0 | 98.9 ± 2.2 | **104.5* ±8.7** | 112.6 ± 13.0 | **123.3 ± 15.2*** |

* Significantly different from control value at $p \leq 0.05$

Mean body weight change data are presented in Tables 3 and 4 for males and females, respectively. Statistically significant ($p < 0.05$) decreases in mean body weight gains were seen in both males and females at 361 mg/kg/day during Weeks 0-6, 6-13, and 0-13 intervals, and in females at 120 mg/kg/day during Week 0-6 interval. No body weight loss was seen in males at 1.2, 18.1, and 120 mg/kg/day, or in the females at 1.2 and 18.1 mg/kg/day groups.

**Table 3. Mean Body Weight Change (G) in Male Rats.**

| Dose Level (mg/kg/day) | Weeks: 0 - 6 | Weeks: 6 - 13 | Weeks: 0 - 13 |
|---|---|---|---|
| 0 | 116.8 ± 5.68 | 55.7 ± 7.4 | 172.6 ± 11.74 |
| 1.23 | 109.8 ± 13.36 | 58.7 ± 7.4 | 168.5 ± 18.3 |
| 18.1 | 113.4 ± 8.66 | 58.7 ± 6.3 | 172.1 ± 11.3 |
| 120.0 | 114.4 ± 11.3 | 58.7 ± 9.0 | 173.1 ± 15.3 |
| 361.0 | **63.1* ± 8.9** | **37.4* ± 10.0** | **100.5* ± 15.2** |

* Significantly different from control at $p \leq 0.05$.

**Table 4. Mean Body Weight Change (G) in Female Rats.**

| Dose Level (mg/kg/day) | Weeks: 0 - 6 | Weeks: 6 - 13 | Weeks: 0 - 13 |
|---|---|---|---|
| 0 | 41.4 ± 5.99 | 28.8 ± 4.3 | 70.2 ± 9.0 |
| 1.2 | 43.9 ± 7.0 | 31.0 ± 5.3 | 74.9 ± 10.6 |
| 18.1 | 43.1 ± 2.3 | 27.0 ± 6.9 | 70.1 ± 8.1 |
| 120.0 | **31.5* ± 4.7** | 30.0 ± 3.4 | 61.4 ± 5.9 |
| 361.0 | **5.4* ± 8.0** | **18.8* ± 8.5** | **24.2* ± 14.1** |

* Significantly different from control at $p \leq 0.05$.

4. Food Consumption

The mean total food consumption was significantly ($p < 0.05$) decreased in males and females at 361 mg/kg/day during Weeks 1-6, 6-13, and 1-13, and in females at 120 mg/kg/day during Weeks 1-6. A significant negative trend was seen for males during Weeks 1-6 and 1-13. No treatment-related changes were seen in males at 1.2,18.1, and 120 mg/kg/day, and in females at 1.2 and 18.1 mg/kg/day groups. The average daily compound consumption for Weeks 1-13 was 1.13, 16.97, 112.87, and 340.97 mg/kg/day for males and 1.15, 17.34, 115.92, and 349.34 mg/kg/day for females.

5. Ophthalmology Examination

Ophthalmoscopic findings for the Week 11 and 13 examinations are summarized in Tables 5 and 6 for males and females respectively. The DMA salt of 2,4-D induced bilateral cataract formation and retinal degeneration in females administered the high dose (361 mg/kg/day). Retinal degeneration was observed in one male rat at this dose; however, this was not supported by collaborative histopathology. No other treatment-related ophthalmoscopic changes were seen in male rats.

**Table 5. Ophthalmoscopic Observations In Male Rats.**

| Observations | Dose Level (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| No.of animals = 10/dose level | 0 | 1.2 | 18.1 | 120 | 361 |
| Conjunctiva - chromodacryorrhea | 1 | 0 | 0 | 2 | 0 |
| Cornea - corneal dystrophy | 1 | 0 | 1 | 0 | 0 |
| Fundus - retinal linear atrophy | 1 | 0 | 1 | 0 | 0 |
| Fundus - possible retinal degeneration | 0 | 0 | 0 | 0 | 1 |
| Coloboma of optic nerve or myopic degeneration | 1 | 0 | 0 | 0 | 0 |

**Table 6. Ophthalmoscopic Observations In Female Rats.**

| Observations | Dose Level (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| No. of animals=10/dose level | 0 | 1.2 | 18.1 | 120 | 361 |
| Conjunctiva - chromodacryorrhea | 0 | 0 | 1 | 0 | 0 |
| Cornea - corneal scar | 1 | 0 | 0 | 0 | 0 |
| Cornea - corneal dystrophy | 0 | 0 | 2 | 0 | 0 |
| Anterior chamber iris - anterior synechia | 1 | 0 | 0 | 0 | 0 |
| Fundus - retinal linear atrophy | 0 | 0 | 1 | 0 | 0 |
| Fundus - retinal degeneration | 0 | 0 | 0 | 0 | 5 |
| Fundus - possible retinal degeneration | 0 | 0 | 0 | 0 | 1 |
| Lens - complete cataract | 0 | 0 | 0 | 0 | 5 |
| Lens - posterior subscapular cataract | 0 | 0 | 0 | 0 | 5 |
| Vitreous/retinas - not visible/examineable | 0 | 0 | 0 | 0 | 3 |

## 6. Hematology and Clinical Chemistry

Treatment-related, statistically significant ($p \leq 0.05$) decreases observed in hematology parameters are summarized below:

| Dose Level / Sex | Parameters Affected | Study Weeks |
|---|---|---|
| 120.0 mg/kg/day (F) | ↓ in RBC | 14 |
| | ↓ in Platelet | 6,14 |
| | ↓ in SEG | 14 |
| 361.0 mg/kg/day (M) | ↓ in RBC | 14 |
| | ↓ in HGB | 14 |
| | ↓ in PLATELET | 6,14 |
| | ↓ in WBC | 6,14 |
| | ↓ in COR WBC | 6,14 |
| | ↓ in LYMPH | 6,14 |
| 361.0 mg/kg/day (F) | ↓ in RBC | 6,14 |
| | ↓ in HGB | 6,14 |
| | ↓ in HCT | 6,14 |
| | ↓ in PLATELET | 6,14 |
| | ↓ in COR WBC | 6,14 |
| | ↓ in SEG | 6,14 |
| | ↓ in LYMPH | 6 |

Alterations observed in hematology parameters correlated with histopathological changes observed in the bone marrow and spleen (see Histopathology).

Treatment-related, statistically significant ( $p \leq 0.05$) differences observed in clinical chemistry parameters are summarized below:

| Dose Level / Sex | Parameters Affected | Study Weeks |
|---|---|---|
| 18.1 mg/kg/day (F) | ↓in $T_3$ | 6 |
| 120.0 mg/kg/day (M) | ↓ in Total protein | 6 |
| | ↓ in Albumin | 14 |
| | ↓ in Calcium | 14 |
| | ↓ in $T_4$ | 6 |
| 120.0 mg/kg/day (F) | ↓ in Glucose | 6 |
| | ↑ in ALT | 6,14 |
| | ↓ $T_3$ | 6,14 |
| | ↓ in $T_4$ | 6,14 |
| 361.0 mg/kg/day (M) | ↓ in Glucose | 6 |
| | ↓ in AST | 6 |
| | ↓ in ALT | 14 |
| | ↓ in Total protein | 6 |
| | ↓ in Globulin | 6,14 |
| | ↓ in Calcium | 6,14 |
| | ↓ in $T_4$ | 6,14 |
| 361.0 mg/kg/day (F) | ↓ in Glucose | 6,14 |
| | ↑ in ALT | 14 |
| | ↓ in Total protein | 14 |
| | ↑ in Albumin | 6 |
| | ↓ in Globulin | 6,14 |
| | ↓ $T_3$ | 14 |
| | ↓ in $T_4$ | 6,14 |

Alterations seen in clinical chemistry parameters correlated well with histopathological changes observed in the liver, thyroids and adrenal glands (see Histopathology).

7. Gross Pathology

Gross necropsy revealed an increased incidence of small testes (7/10) and soft testes (3/10) in males and opaque eyes (6/10) in females at the high dose (361 mg/kg/day). Other gross necropsy findings were considered incidental in nature.

8. Organ Weights

Statistically significant differences in absolute and relative organ weight were primarily seen in male and/or female rats at the high dose. The absolute weights of adrenals (females), thymus (both sexes), brain (both sexes), heart (both sexes), kidneys (males), testes with epididymides, ovaries and pituitary (females) were decreased while absolute liver weights were increased in females. The relative weights of adrenals (males), brain (both sexes), heart (both sexes), kidneys (both sexes), liver (both sexes), thyroid/parathyroid (both sexes), and pituitary (males) were increased while the relative weights of testes with epididymides, ovaries, and pituitary (females) were decreased.

9. Histopathology

Treatment-related histopathological changes (Tables 7 and 8) were primarily observed in the high dose group which included:

1. Hypertrophy of the zona glomerulose of the adrenal cortex,
2. Bilateral retinal degeneration and cataract formation of the eye
3. Hypertrophy of thyroid follicular cells,
4. Centrilobular hepatocellular hypertrophy of the liver,
5. Brush border loss in proximal tubular cells of kidneys,
6. Atrophy of the testes,
7. Hypoplasia of bone marrow, and
8. Hypoplasia of spleen.

Histopathological evaluation of the eye revealed degenerative changes in the lens and retina in females at the high dose. Retinal degeneration was slight in one, and moderate in six animals. Cataractous change was moderate in one animal and moderately severe in five. In most cases, the histopathological changes correlated well with alterations observed in hematology and clinical chemistry parameters and organ weight data. The increase in adrenal gland weight can be correlated to hypertrophy of cells in zona glomerulose. Hypertrophy of follicular cells of the thyroid reflects a response to decreased circulating thyroid hormone levels. Increases in liver weight, ALT, and AST are associated with centrilobular hepatocellular hypertrophy of the liver. Increased kidney weights (females) correlated with brush border loss in the proximal tubules. Decreases in testes weight correlated with testicular atrophy, while decreased ovarian weights did not show any corresponding histological changes. Decreases in RBC, HGB and HCT are associated with bone marrow hypoplasia. Decreases in absolute and corrected leukocyte counts correlated with lymphoid of the spleen and bone marrow. Other microscopical changes were considered incidental and unrelated to treatment.

**Table 7. Histopathological Findings in Male Rats At Sacrifice.**

| Organ / Lesion | Dose Level (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| No.of animals= 10/dose levels | 0 | 1.2 | 18.1 | 120 | 361 |
| Adrenal, cortex -Hypertrophy, zona glomerulose | 0 | 0 | 0 | 0 | 10 |
| Thyroid -Follicular cell hypertrophy | 0 | 0 | 0 | 0 | 4 |
| Kidney -Brush border loss, tubular | 0 | 0 | 0 | 0 | 7 |
| Testes -Atrophy, bilateral | 0 | 0 | 0 | 0 | 10 |
| Marrow, femur -Hypoplasia | 0 | 0 | 0 | 0 | 7 |
| Eye -Corneal dystrophy | 2 | 1 | 5 | 3 | 6 |

**Table 8. Histopathological Findings in Female Rats At Sacrifice.**

| Organ / Lesion | Dose Level (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| No.of animals= 10/dose levels | 0 | 1.2 | 18.1 | 120 | 361 |
| Adrenal, cortex -Hypertrophy, zona glomerulose | 0 | 0 | 0 | 0 | 8 |
| Thyroid -Follicular cell hypertrophy | 0 | 0 | 0 | 0 | 6 |
| Kidney -Brush border loss, tubular | 0 | 0 | 0 | 0 | 8 |
| Liver - Hypertrophy | 0 | 0 | 0 | 0 | 7 |
| Marrow, femur -Hypoplasia | 0 | 0 | 0 | 0 | 9 |
| Spleen - Hypoplasia | 0 | 0 | 0 | 0 | 7 |
| Eye | | | | | |
| -Corneal dystrophy | 1 | 1 | 2 | 3 | 2 |
| -Degeneration, retinal, bilateral | 0 | 0 | 0 | 0 | 7 |
| -Cataractous change, bilateral | 0 | 0 | 0 | 0 | 6 |

## IV. DISCUSSION

Male and female Fischer-344 rats were fed diets containing DMA salt of 2,4-D at concentrations of 0, 1.2, 18.1, 120.0, or 361.0 mg/kg/day for 13 weeks.

Analytical data showed that the diet mixes were homogeneous, stable at room temperature for up to 14 days and the mean concentrations were within 10% of the target level.

Treatment had no effect on survival. No treatment-related effects were seen at the low- (1.2 mg/kg/day) or the mid-1- dose (18.1 mg/kg/day). Decreases in mean body weight, body weight gain, and reduction in food consumption were observed in rats fed the mid-2- (120 mg/kg/day) and the high-dose (361 mg/kg/day).

Ophthalmology examinations revealed bilateral cataract formation and retinal degeneration only in females at 361.0 mg/kg/day; no ocular changes were seen at the lower doses.

Treatment-related alterations in hematology parameters were confined to the 120 mg/kg/day and 360 mg/kg/day groups which included: decreased red cell mass, decreased hemoglobin, decreased platelet count, decreased absolute and corrected leukocyte counts, decreased lymphocyte counts, and decreased segmented neutrophil counts. Changes in cell morphology consisted of decreased incidence/grade for echinocytes and acanthocytes.

Also, treatment-related alterations in clinical chemistry parameters were primarily seen at 120 and 360 mg/kg/day groups which included: decreased glucose and globulin levels decreased thyroxin levels, decreased triiodothyronine, decreased total protein, increased ALT, and increased albumin levels. The significant decrease in thyroxine level seen in females at 18.1 mg/kg/day (2.3 µg/DL vs 3 µg/DL in controls) at Week 6 was within the reference range (2.2 to 5.6 µg/DL) and therefore, was not considered to be treatment related.

Treatment-related gross pathological changes were limited to small and soft testes (males) and opaque eyes (females) at the high dose. Compound-induced differences in absolute and relative organ weights were generally limited to the high-dose which included: decreased mean absolute brain weight and increased mean brain/body weight rations, increased mean relative thyroid weights, decreased mean absolute kidney weight and increased mean kidney/body weight ratio, and increased absolute and relative liver weights.

In most cases, alterations noted in hematology, clinical chemistry, gross pathology, and organ weights correlated well with histopathological changes. Treatment-related histopathological changes which were limited to the high-dose group included bilateral retinal degeneration and cataract formation (females), centrilobular hepatocellular hypertrophy (females), atrophy of the testes (males), hypertrophy of thyroid follicular cells, and brush border loss in proximal tubular cells in the kidney (males and females), and hypoplasia of the spleen (females) and bone marrow (males and females).

## V. CONCLUSION

Under the conditions of this study, a NOEL of 18.1 mg/kg/day and a LOEL of 120.0 mg/kg/day is established for the 90-day oral toxicity of the Dimethylamine Salt of 2,4-Dichlorophenoxyacetic acid to male and female rat. The LOEL is based on decreases in mean body weight, body weight gain and food consumption, and alterations in hematology and clinical chemistry parameter observed at 120 mg/kg/day.

VI. **CORE CLASSIFICATION:** Guideline; this study satisfies the requirement (82-1a) for a 90-day feeding study in rodents.

# END